Plasma Paracetamol Concentrations Measured by Fluorescence Polarization Immunoassay and Gastric Emptying Time

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KOIZUMI, F., KAWAMURA, T., ISHIMORI, A., EBINA, H. and SATOH, M. *Plasma Paracetamol Concentrations Measured by Fluorescence Polarization Immunoassay and Gastric Emptying Time.* Tohoku J. exp. Med., 1988, **155** (2), 159-164 — Radionuclide labeled gastric emptying was compared with plasma paracetamol (acetaminophen) concentration measured by fluorescence polarization immunoassay. A significant correlation was found between both gastric emptying half-time of radionuclide ($t_{1/2}$) and % isotope remained in the region of the stomach and paracetamol absorption kinetic parameters. The strongest correlation was found between $t_{1/2}$ and the time required to reach the peak plasma level of paracetamol ($r=0.680$, $p<0.01$). This study indicates that simple paracetamol marker method has enabled an indirect quantitative measurement of gastric emptying by sampling at varying time intervals. ——— paracetamol; gastric emptying; fluorescence polarization immunoassay

Over the past few years, several disorders of gastric motility leading to a delayed or rapid gastric emptying have been reported (Minami and McCallum 1984; Horowitz et al. 1985), and thus the use for measuring gastric emptying is essential for understanding physiological and clinical problems associated with those disorders. In these years a variety of methods have been developed to measure the rate of gastric emptying. The general methods currently used for evaluating gastric emptying are (1) intubative techniques (Hunt and Spurrell 1951; Goldstein and Boyle 1965; George 1968), (2) radiologic techniques (Canon 1902; Abe 1981), (3) radioisotopic techniques utilizing external scanning (Griffith et al. 1966; Chaudhuri 1974; Horowitz et al. 1982), and (4) ultrasound techniques (Bateman and Whittingham 1982). The use of blood concentration of paracetamol, which is not absorbed from the stomach but rapidly from the small bowel, has also been used as an indirect measure of the rate of gastric emptying (Heading et al. 1973; Nimmo et al. 1975; Harasawa et al. 1979; Goldstraw and

Bach 1981; Nakanome et al. 1982; Ogoshi et al. 1982; Marsh et al. 1984; Maddern et al. 1985).

Spectrophotometric method (Harasawa et al. 1979; Nakanome et al. 1982; Ogoshi et al. 1982), gas-chromatography (Heading et al. 1973; Nimmo et al. 1975; Goldstraw and Bach 1981) and high-pressure liquid chromatography (Marsh et al. 1984; Maddern et al. 1985) have been used exclusively as a technique of choice for determining paracetamol in plasma. The disadvantage of each method such as requiring much time and efforts has also been pointed out. Recently, a reliable, simple and rapid assay for paracetamol based on the principle of fluorescence polarization immunoassay (FPIA) has been available, and therefore we compared radionuclide-labeled gastric emptying with plasma paracetamol concentration.

MATERIALS AND METHODS

The study subjects included 7 patients with gastritis, 11 patients with gastric ulcer, 6 patients with duodenal ulcer and 1 patient with gastric cancer. Informed consent was obtained from all subjects. The gastric emptying test was performed after an overnight fast. The test procedures used were described previously (Koizumi et al. 1987): Briefly, a standard, Japanese style hospital lunch for patients comprising rice water, bean or miso-soup, a raw egg and a lactic acid beverage (25 g carbohydrate, 6.5 g fat and 10.2 g protein, 206 Kcal) and 1.5 g paracetamol was prepared. In the rice water 1 mCi dose of ^{99m}Tc -Sn colloid was mixed. Meal and paracetamol were consumed within 5 min. Immediately thereafter, the amount of isotope remaining in the stomach was determined at 15-min intervals for 65 min using a gamma camera (LFOV, Shimadzu, Kyoto) and results were recorded on videotape. After the area of interest was obtained based on the recorded data, the gastric emptying rate was determined by Scinti-pac 230 (Shimadzu) from the decreasing intragastric fraction of the total radioactivity determined at sequential scanning periods and

Fig. 1. Standard curve showing the relationship between the decreasing polarization of fluorescence and increasing levels of added paracetamol in serum.

TABLE 1. *Precision of paracetamol concentration by assaying three control sera 10 times in one assay and in 10 consecutive assays*

Target level ($\mu\text{g/ml}$)	Measured level mean $\pm$ s.d. ($\mu\text{g/ml}$)	% CV	% Bias
Within-assay precision			
6.25	5.6 ± 0.7	12.1	-10.4
12.5	12.6 ± 0.4	3.2	0.8
25	26.0 ± 0.8	2.9	4.0
Between-assay precision			
6.25	6.0 ± 0.4	7.1	-4.0
12.5	13.4 ± 1.1	7.9	7.2
25	26.2 ± 0.9	3.5	4.8

s.d., standard deviation ; CV, coefficient of variation ; % Bias = $100 \times (\text{measured concentration} - \text{target concentration}) / \text{target concentration}$

the half-life ($t_{1/2}$) of the meal in the stomach was calculated. Counts were taken in the standing position and subjects were permitted to move freely during counting.

Blood samples were taken at -30 min before meal and at 5, 20, 35, 50, 65 and 95 min after ingestion for assessing paracetamol absorption parameters.

Paracetamol concentrations in plasma were estimated in duplicate with a FPIA system (TDX[®], Dainabot, Irving, TX, USA). The principles of FPIA have been reviewed (Jolley 1981). Fig. 1 shows a typical assay standard curve of paracetamol in which the polarization in arbitrary units of fluorescein-labeled paracetamol bound to the antibody is plotted against the concentration of paracetamol in microgram per milliliter.

Paracetamol control samples for precision studies were prepared by dissolving paracetamol 200 mg in 200 ml methanol and were serially diluted to 6.25, 12.5 and 25 $\mu\text{g/ml}$ with drug-free human serum. The precision data obtained are summarized in Table 1. Intra- and inter-assay reproducibility was good.

Coefficients of the correlations (r) between the $t_{1/2}$ of radionuclide and plasma paracetamol concentrations measured at the 6 different postdose times, peak concentration and time to peaked concentration (t_{max}), and between the % isotope retention and the area under the plasma paracetamol concentration-time curves (AUC) up to 20, 35, 50 and 65 min postdose were calculated by the least-squares regression analysis.

RESULTS

The mean isotope remaining in the stomach was $75.6 \pm 10.8\%$, $54.5 \pm 17.8\%$, $37.7 \pm 16.7\%$ and $27.8 \pm 16.0\%$ at 20, 35, 50 and 65 min, respectively. The respective mean paracetamol concentrations were $4.1 \pm 6.0 \mu\text{g/ml}$, $10.7 \pm 8.6 \mu\text{g/ml}$, $14.6 \pm 8.2 \mu\text{g/ml}$, $15.7 \pm 7.4 \mu\text{g/ml}$, $15.4 \pm 6.4 \mu\text{g/ml}$ and $14.0 \pm 4.8 \mu\text{g/ml}$ at 5, 20, 35, 50, 65 and 95 min. The mean peak plasma paracetamol concentration occurred at 50 min after the oral dosing (Fig. 2).

The $t_{1/2}$ value of gastric emptying time derived from the isotope technique was compared with the plasma concentrations of paracetamol measured at 5 to 95 min postdose and the time required to reach the peak. As listed in Table 2, a significant correlation was found between the $t_{1/2}$ value for radionuclide and both

Fig. 2. Gastric emptying rate (A) and paracetamol concentration-time course (B) in 25 subjects. Counts at 5 min after meal were normalized to 100% (A). Results are shown in terms of mean $\pm$ S.D.

TABLE 2. Relationship between gastric emptying $t_{1/2}$ measured with radionuclide technique and paracetamol absorption parameters, and between % isotope retention and AUC values ($\mu\text{g} \cdot \text{hr/ml}$) calculated up to 65 min postdose

	r	p
$t_{1/2}$ vs. plasma paracetamol concentration at time		
5	0.119	n.s.
20	-0.300	n.s.
35	-0.414	<0.05
50	-0.232	n.s.
65	-0.096	n.s.
95	0.204	n.s.
$t_{1/2}$ vs. peak plasma paracetamol concentration	-0.163	n.s.
$t_{1/2}$ vs. time of peak plasma paracetamol	0.680	<0.01
% Isotope retention at time		
20 vs. AUC_{0-20}	-0.304	n.s.
35 vs. AUC_{0-35}	-0.519	<0.01
50 vs. AUC_{0-50}	-0.398	<0.05
65 vs. AUC_{0-65}	-0.352	n.s.

n.s., not significant.

plasma paracetamol level at 35 min after meal ($r = -0.414$, $p < 0.05$) and the time required to reach the peak ($r = 0.680$, $p < 0.01$). When the percent count of isotope in the stomach was compared with the AUC, there was a significant correlation between percent count at 35 min and AUC from 0 to 35 min ($r = -0.519$, $p < 0.01$), and percent count at 50 min and AUC from 0 to 50 min ($r = -0.398$, $p < 0.05$).

DISCUSSION

FPIA becomes increasingly popular for plasma drug determination because of its rapidity, simplicity, specificity and reproducibility. Only 50 μ l of plasma is needed and it does not require extraction as in previously established paracetamol assay methods (Heading et al. 1973; Nimmo et al. 1975; Harasawa et al. 1979; Goldstraw and Bach 1981; Nakanome et al. 1982; Ogoshi et al. 1982; Marsh et al. 1984; Maddern et al. 1985).

There were considerable interindividual variations in the rate at which orally administered paracetamol was absorbed in this study. The peak plasma concentration of paracetamol varied from 6.4 μ g/ml to 36.9 μ g/ml and the time required to reach peak concentrations ranged from 20 to 95 min after ingestion. Significant correlation exists between both gastric emptying $t_{1/2}$ measured with the radionuclide technique and the % count in the stomach and paracetamol absorption parameters. The statistically strongest correlation was found between the $t_{1/2}$ and $t_{\max}$. The absorption of paracetamol is, therefore, likely to be dependent on the rate of gastric emptying.

Previously reported studies have failed to take frequent samples of plasma, and the plasma paracetamol concentration at 45 min after ingestion was used as the indirect indicator of gastric emptying rate (Harasawa et al. 1979; Nakanome et al. 1982; Ogoshi et al. 1982). Results obtained in this study verify that the time required to reach the peak as measured by frequent blood sampling reflects more precisely the radionuclide-assessed gastric emptying time.

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